REMARKS

It is Applicants' understanding that all previous rejections and objections have been resolved excpt for the obviousness rejection under 35 USC 103(a) based upon Sisler, US Patent 6,194,350; Daly, et. al., US Patent 6,017,849; and Minkin (1997, IDS Reference) and the double patenting rejections (which will be resolved upon approval by the Office of the previously filed Terminal Disclaimers).

The following comments relate to the rejections detailed in the Office Action.

Rejection under 35 USC § 103(a)

Claims 1 and 11 are rejected under under 35 USC § 103(a) as being unpatentable over Sisler, E., US Patent No. 6,194,350 ("'350"), Daly, et al., US Patent 6,017,849 ("'849"), and Minkin, et al., *Theochem, NL*, 1997, 398-399, pp. 237-253 ("Minkin") in that all the references teach cyclopropene derivatives and methods of blocking ethylene receptors in plants. The Office Action stated that in their prior Response, Applicants' had not overcome this rejection, partially because Applicants had addressed each reference individually, not the references in combination.

It is still Applicants' position that Minkin is not a valid reference for obviousness, either alone or in combination with '350 and '849. Minkin presents a completely different problem and solution from those addressed by '350 and '849. '350 and '849 relate to the use of cyclopropenes to inhibit the ethylene response in plants while Minkin relates to computational modeling of the mechanisms of circumambulatory rearrangements of main-group migrants (that is, substituents) in the cyclopropene ring. There is no disclosure, teaching, or suggestion of biological activity of any kind in Minkin. Minkin is concerned with the various mechanistic factors related to substituent group migrations in the cyclopropene ring and comparison of those factors with substituent group migrations in cyclopentadienes (see the Abstract; page 238, first full paragraph; page 251, Conclusions). In addition, Minkin does not actually disclose the synthsis of any compound discussed. Rather, the reference is limited to computational modeling of hypothetical compounds (see the Abstract; page 238, first column, line 22 to end of paragraph; page 238, Methods; page 239 first column, lines 8-11; page 243, first column, lines

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18-21; page 247 first column, lines 4-7 and second column). Therefore, Minkin should not be considered as being an enabling reference as it does not describe the synthesis of any particular cyclopropene nor their use for any purpose other than computational, mechanistic studies. In order to advance prosecution of this Application, Applicants have, in their prior response, added the dithioformyl group (the substitutent in compounds 13a, 13b, 13c, and 13d) to those substituent groups which are disclaimed (see *In re Johnson and Farnham*, 194 USPQ 187, 196 (1977).

The Minkin compounds specifically noted by the Examiner; that is, compound 2b in Scheme 1 on page 239 and compounds 13a, 13b, 13c, and 13d on page 247 are not claimed by Applicants. Compound 2b is a tetrasubsituted cyclopropene (three phenyl groups and an -NCS or -SCN group) whereas Applicants' claimed compounds require at least two hydrogen substituents. Compounds 13 a-d are now disclaimed. In any case, however, due to the non-analogus nature of Minkin, Applicants' compounds would not be obvious to one skilled in the art based upon this reference. There is no disclosure, teaching, or suggestion in Minkin that would motivate one skilled in the art who was seeking compounds to inhibit the action of ethylene in or on plants to prepare Applicants' claimed compounds.

'350 discloses a method of inhibiting an ethylene response in a plant comprising applying to the plant an effective ethylene response-inhibiting amount of a cyclopropene containing from 1 to 4 R groups wherein each R is independently a C6-C20 alkyl, alkenyl, or alkynyl. '350 further defines "alkyl, alkenyl, and alkynyl" as follows: "...one or more of the carbons in one or more of the R groups is replaced by a group such as ester groups, nitriles, amines, amine salts, acids, acid salts, esters of acids, hydroxyl groups, halogen groups, and heteroatoms selected from the group consisting of oxygen and nitrogen or where such chains include halogen, amino, alkoxy, carboxy, alkoxycarbonyl, oxycarbonylalkyl, or hydroxy substituents." In essence, what this means is that each R group must contain a minimum of six non-hydrogen atoms which are selected from the group consisting of carbon, oxygen, nitrogen, or halogen. Those substituents of the cyclopropene ring which are not R groups **must** be hydrogen.

However, '350 also teaches that examples of the preferred compounds of their invention include hexyl, heptyl, octyl, nonyl, and decyl substituents and that the alkyl groups are

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preferably linear and saturated (see col. 2, lines 44-46). All of the working examples of '350 are linear alkyl substituted 1-cyclopropenes, including the comparative examples. Therefore, one skilled in the art, who was attempting to prepare alternatives to those compounds disclosed in '350 would not be motivated to prepare Applicants' compounds, which are cyclopropenes with complex substituent groups which include heterocyclic or carbocyclic rings, substituents with atoms not disclosed, taught, or suggested by '350 (such as, for example, silicon, sulfur, boron, and phosphorous), or compounds with multiple substituent patterns. On the contrary, one skilled in the art seeking to find ethylene inhibiting cyclopropenes would be motivated by the teachings in '350 to seek compounds with linear alkyl groups. There is no disclosure, teaching, or suggestion in '350 that would motivate one skilled in the art in the direction of Applicants' claimed compounds

'849 discloses encapsulated cyclopropene derivatives in which the cyclopropene is substituted with from 1 to 4 of hydrogen, saturated or unsaturated C1-C4 alkyl, hydroxy, halogen, C1 to C4 alkoxy, amino, and carboxy. The cyclopropene compounds are the same as those previously disclosed in an earlier patent by Sisler, US Patent No. 5,518,988, which discloses ethylene response antagonistic cyclopropenes substituted with from 1 to 4 of C1 to C4 saturated or unsaturated alkyl, hydroxy, halogen, alkoxy, amino, and carboxy. The difference between '350 and '849 is that '849 teaches encapsulation of the cyclopropenes originally disclosed in Sisler's US Patent No. 5,518,988. Thus, this reference discloses and teaches that cyclopropenes comprising a certain, and again, limited, group of small substituents can be encapsulated and then used as effective ethylene antagonists.

Furthermore, '849 teaches that preferred compounds are cyclopropene (that is, an unsubstituted compound), dimethylcyclopropene, and methylcyclopropene (see col. 6, lines 37-46; col. 7, lines 49-50 and 61; and claims 3 and 6). Most preferably, the alkyl groups are single carbon or linear (see col. 9, lines 42-43). All the working examples in '849 utilize 1-methylcyclopropene. Therefor, one skilled in the art seeking to find ethylene inhibiting cyclopropenes would be motivated by the teachings in '849 to those compounds with small substitutent groups and, preferably, linear alkyl groups. There is no disclosure, teaching, or suggestion in '849 that would motivate one skilled in the art in the direction of Applicants' claimed compounds.

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It should be noted that in an earlier publication by Sisler, *Physiologia Plantarum* 100: 577-582, 1997 (copy of which is included in this response), the authors note the huge difference in biological activity between a cyclopropene with two small alkyl substituent groups (3,3-dimethylcyclopropene) when compared to an unsubstituted cyclopropene and a monosubstituted cyclopropene (1-methylcyclopropene). The authors found that the disubstituted cyclopropene was approximately 1000x less active than the unsubstituted or monosubstituted cyclopropene (see page 578, Table 1). This referrence, by the inventor of '350, supports Applicants' position that one skilled in the art would not be motivated to substitute the cyclopropene ring with the more complex substituent groups and multiple substituent groups claimed by Applicants. Applicants' data found in Table 3 of the Application shows results that differ significantly from the pattern observed by Sisler for cyclopropene, 1-methylcyclopropene, and 3,3-dimethylcyclopropene. See, in particular, compounds 17 and 18 vs. compounds 19 and 20 and compound 8 vs. compound 17.

In combination, Minkin, '350, and '849 provide no more motivation to one skilled in the art to prepare Applicants' claimed compound than do each of these references separately. '350 is an extension of the disclosure of '849 in that it discloses and teaches ethylene antagonistic cyclopropenes containing from 1 to 4 of C6 to C20 alkyl, alkenyl, and alkynyl groups substituted with a very similar group of substituents as those disclosed in '849. However, the substituent groups again are quite limited in scope. Both references teach that preferred substituent groups are linear alkyl groups. In both references all of the working and comparative examples are cyclopropenes substituted with linear alkyl groups. One of ordinary skill in the art and familiar with these references would note that the cyclopropene substituent groups disclosed in '350 and '849 are not significantly different than those disclosed in the original Sisler patent (US 5,518,988), that is, that they are linear alkyl groups and, therefore, would conclude that active cyclopropenes must contain **only** such substituent groups.

As noted above, Minkin provides no motivation to one skilled in the art to modify the compounds disclosed in '350 and '849 to provide ethylene inhibitory compounds. In fact, Minkin provides no motivation at all to prepare any cyclopropene compounds. Minkin is concerned only with the various calculated energy states of substituted cyclopropenes as they undergo various rearrangements of substitutent groups around the cyclopropene ring.

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Applicants, on the other hand, have discovered that cyclopropenes with ethylene inhibition activity can contain substituent groups, or substituent group patterns, which are significantly different from those disclosed, taught, or suggested by '350 and '849, as well as Minkin. These very different substituent groups are selected from, a 4 to 14 membered carbocyclic or heterocyclic ring system; certain silicon, sulfur, phosphorous, or boron-containing groups; and a combination of large and small substituent groups. There is no disclosure, teaching, suggestion, or motivation in '350, '849, and/or Minkin, either alone or in combination, that would lead one skilled in the art to Applicants' claimed cyclopropenes. Therefore, the subject matter as a whole of Applicants' claims would not have been obvious to a person having ordinary skill in the art with a knowledge of '350, '849, and Minkin.

With this response, Applicants believe that the rejections have been overcome and the claims are in condition for allowance. Should the Examiner have any suggestions which may put the Application in better condition for allowance, Applicants' attorney is willing to discuss any such suggestions either by phone or at the U. S. Patent and Trademark Office.

Respectfully submitted,

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Inhibitors of ethylene responses in plants at the receptor level: Recent developments

E. C. Sisler and M. Serek

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A number of organic molecules that appear to block the ethylene receptor have been discovered recently. For example, on irradiation with visible light, diazocyclopentadiene (DACP), gives rise to some potent but as yet unidentified inhibitor compounds. Some synthetic cyclopropenes have been shown to bind to the ethylene receptor and prevent the physiological action of ethylene for extended periods. Cyclopropene (CP), 1-methylcyclopropene (1-MCP) and 3,3-dimethylcyclopropene (3,3-DMCP) have been shown to prevent ethylene effects in a number of plants. As low a concentration as 0.5 nl l^{-1} of 1-MCP is sufficient to protect carnation (*Dianthus caryophyllus*) flowers for several days against ethylene, and 0.7 nl l^{-1} 1-MCP or CP will prevent the ripening of banana (*Musa sapientum*) for 12 days at 24°C. Some plant organs require higher concentrations of these inhibitors. Complete inhibition of ethylene effects in pea seedlings requires treatment with 40 nl l^{-1} of 1-MCP. These novel inhibitors appear to be suitable for many commercial applications including extending the vase life of cut flowers and the display life of potted plants. Since 1-MCP apparently is non-toxic at concentrations that are active, it may in future be available for regulating the ripening of fruits and preventing the deleterious effects of ethylene in vegetables.

Key words – CP, cyclopropene, DACP, diazocyclopentadiene, 3,3-DMCP, 3,3-dimethylcyclopropene, ethylene action inhibitors, ethylene receptor, 1-MCP, 1-methylcyclopropene.

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Introduction

The potent effects of ethylene on plant growth and development were first discovered at the turn of the century (Neljubov 1901). Although much has since been learned about the role of this simple plant hormone, its mode of action is still imperfectly understood. Early on, it was shown that other compounds such as carbon monoxide, acetylene, and propene had similar activities, but at much higher concentrations than ethylene. Carbon dioxide was also shown long ago to be an antagonist of ethylene; much research points to the ethylene receptor as the site of this inhibition but this has not been verified.

In 1967, Burg and Burg, based on a correlation between the relative ethylene-like activity of a number of compounds and their known order of binding to silver ion, postulated the presence of a metal in the ethylene receptor. Although no metal has yet been shown to be present, the information obtained so far is best explained by this hypothesis. Sisler and Pian (1973) reported that 2,5-norbornadiene counteracted ethylene in a competitive manner. Since then other compounds, e.g. *trans*-cyclooctene, have been shown to counteract ethylene by interacting with the receptor but, like 2,5-norbornadiene, require continuous exposure, a high concentration, and have a strong odour (Sisler and Yang 1984, Sisler 1991). Beyer (1976) reported that silver ion negated the effects

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of ethylene. The effect was considered to be non-competitive. Silver, applied in the form of thiosulfate, is a very effective inhibitor of ethylene responses, but as it is a heavy metal it cannot be used on food and feed, and has been objected to by environmentalists. It has been used with much success on cut flowers and potted plants. How silver acts is not known for certain, but it may also act at the receptor level (Veen 1986).

Recently, organic molecules that block the ethylene receptor for extended periods have been discovered. Some of these render tissues insensitive to ethylene for 10–12 days and act at concentrations as low as 0.5 nl l^{-1}.

In this paper we review the current status, probable mode of action, and future prospects for the use of these compounds. Much of the earlier work on the ethylene receptor has been reviewed by Sisler (1991).

Abbreviations – CP, cyclopropene; DACP, diazocyclopentadiene; 3,3-DMCP, 3,3-dimethylcyclopropene; 1-MCP, 1-methylcyclopropene; STS, silver thiosulfate.

Diazocyclopentadiene

Diazocyclopentadiene (DACP) (Sisler and Blankenship 1993a,b, Sisler et al. 1993) is a weak inhibitor of ethylene responses, but upon irradiation with visible light gives rise to one or several much more active components that block ethylene responses for many days. At 25°C, this blockage lasts about 10–12 days in *Lycopersicon esculentum* (tomato) fruits, and at 14.5°C, it is about twice that long. The active component in irradiated DACP has not been identified but the products appear to be very unstable. A major problem with DACP is that it is explosive in high concentrations, which limits its commercial usefulness. Products from the irradiation of DACP have been shown to inhibit the effects of ethylene in fruits of banana, kiwifruit (*Actinidia deliciosa*), persimmon (*Diospyros kaki*), avocado (*Persea americana*) (E. C. Sisler and N. Lallu, unpublished results), tomato (Sisler and Blankenship 1993b, Sisler and Lallu 1994) and several ornamentals including carnation, geranium (*Pelargonium zonale*) and rose (*Rosa hybrida*) (Serek et al. 1993, 1994a).

Cyclopropenes

Cyclopropenes (Sisler et al. 1996a,b) have been found to be effective antagonists of the ethylene response. CP, 1-MCP and 3,3-DMCP are all active, but CP and 1-MCP are about 1 000 times more active than 3,3-DMCP (Tab. 1). All of these are gasses at room temperature and have no obvious odour at the concentrations needed to protect plants. Most of the studies to date have been done with 1-MCP, since it is more stable than CP and more active than 3,3-DMCP.

Preparation and physical properties

1-MCP is the most useful compound among recently developed inhibitors of ethylene responses. It is more stable than CP and is active at a considerably lower concentration, and providing protection for a longer time than 3,3-DMCP. It is not yet available commercially, thus details of its synthesis will be given here. Adapted from the method of Magid et al. (1970), the following procedure provides a preparation of 1-MCP suitable for most experimental purposes.

Tab. 1. Minimum concentration and time of insensitivity in *Musa sapientum* fruits.

Compound	Concentration, nl l^{-1}	Insensitivity, days
1-MCP (1-methylcyclopropene)	0.5	12
CP (cyclopropene)	0.5	12
3,3-MCP (3,3-dimethylcyclopropene)	500	7

Synthesis of 1-MCP

Place a rubber stopper in a test (or other) tube and pull a high vacuum through a hypodermic syringe to remove the air. Flame the tube while the vacuum is being pulled to drive out moisture. Allow an inert gas (helium, argon or nitrogen) that is free of oxygen and water to enter. Repeat twice. With a hypodermic syringe that has been flushed with inert gas remove and inject 5 ml of 1.8 *M* solution of phenyllithium (Aldrich Chemical Co., Milwaukee, WI, USA) in solvent of 70% cyclohexane and 30% ether. Inject inert gas as the phenyllithium is removed from its container to prevent air from entering. Allow the excess gas pressure in the tube to be relieved. Over about 30 min inject 0.3 ml of 3-chloro-2-methylpropene (Aldrich Chemical Co.). As 1-MCP is formed, white lithium chloride will precipitate and the lithium salt of 1-MCP will be in solution. This is stable in the freezer (–20°C) for months.

To prepare 1-MCP, withdraw a sample through the stopper and inject it into a sealed container containing some water. Aldrich Sure Seal bottles are convenient but not necessary. After warming and shaking, excess organic solvent can be withdrawn through the stopper. Inject some saturated ammonium sulfate solution to form a seal. Store inverted. The concentration of 1-MCP in the tube can be determined by gas chromatography using a hydrocarbon separation column other than alumina (an alumina GC column destroys 1-MCP). There will be three major peaks if ether-cyclohexane is used as solvent. 1-MCP will be the first peak, which can be calibrated against butane (or possibly ethylene). The preparation contains some impurities, but as it contains so much activity the impurities usually can be ignored.

Biotechnologies for Horticulture Inc., 122 Tower Drive, Burr Ridge, IL 60521-5795, USA, is expected to market commercial preparations of 1-MCP during 1997.

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Other syntheses

CP can be prepared from 3-chloropropene (allyl chloride) by adding this compound dropwise to sodium amide in mineral oil at 80°C (Closs and Krantz 1966). 3,3-DMCP can be prepared by the method of Binger (1974). DACP can be prepared by published methods (Regitz and Liedhegener 1967), but caution should be exercised because of its explosive nature in concentrated form. The concentration can be determined by the use of triphenylphosphine (Ramirez and Levy 1958).

Stability of compounds

DACP can be kept in the dark at –80°C. CP is unstable even at –80°C but can be stored at –196°C. At low concentrations (about 1%), it can be stored in the gas phase at room temperature for a few weeks under nitrogen, but some decomposition occurs with time (Hopf et al. 1985). 1-MCP can be stored when diluted in an inert gas phase at 1.33 kPa at room temperature (Bailey and Walsh 1978). It is, however, unstable in the liquid phase even at –20°C. Thus 1-MCP should be free of other liquid organic solvents. 3,3-DMCP is very stable, even at 100°C.

Physical properties

DACP is a liquid with a boiling point of 30°C at 4 kPa. Because of its explosiveness, it is usually not distilled. It should be kept at 25% solution in a solvent such as pentane. CP boils at –35°C with much loss due to polymerization. It can polymerize resulting in explosiveness if warmed too rapidly even in solutions. The boiling point of 1-MCP is considered to be 10°C or lower. 3,3-DMCP is reported to boil at 14.5°C. Thus, all of these compounds are gasses at room temperature (Closs 1966).

Treatment of plants

Any air-tight container is satisfactory for treatment. The concentration required to protect plants depends on the time of exposure. The longer the exposure, the lower the required concentration (Sisler et al. 1996a). A plot of the logarithm of the concentration vs that of time of exposure required to inactivate the receptor gives a straight line in carnation (Fig. 1). There is a considerable difference in the amount of 1-MCP required for protection in different plants (Tab. 2): 1-MCP completely protects carnation and banana by a 24-h exposure at 0.5 nl l^{-1}. That means 15 ml of pure gas should be sufficient to treat 30 000 m^3 of space. The volume of the container used is usually 3 l per banana, but no detailed study has been made on the importance of container volume. In tomato fruits obtained commercially at the mature green stage, a higher level is required – treatment with 7 nl l^{-1} 1-MCP for 24 h is needed for complete protection. For maximum response in growing vegetative tissue and in abscission, a higher concentration is needed. Treatment

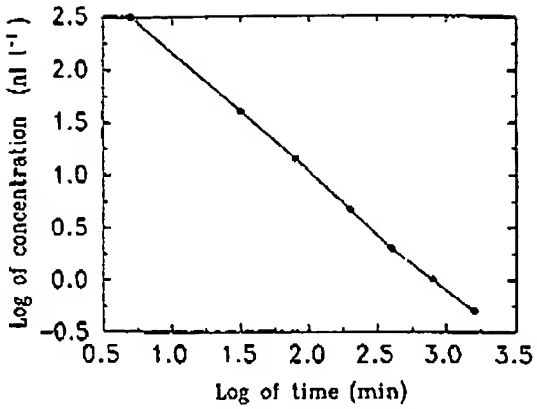

Fig. 1. Log concentration vs log time in inactivating the receptor in *Dianthus caryophyllus* flowers.

with 40 nl l^{-1} of 1-MCP for 6 h is required for maximum retardation of pea seedling growth. It is unclear why a much higher concentration is needed to counteract ethylene in these tissues, but even this higher level represents a far lower amount of active substance than of ethylene required to elicit the normal response. It will be interesting to study the basis of the large differences in the 1-MCP concentration required to inactivate the receptor in different plant species and, perhaps, tissues.

For how long time are plants protected?

The length of the protection period has not yet been determined accurately. Some flowers may deteriorate for other reasons before they again become ethylene sensitive, thus preventing an accurate determination. Carnations treated with 1-MCP appear to remain insensitive for 12–15 days at 24°C. Some plant parts may become infected by fungi before they become sensitive to ethylene after treatment with inhibitors. Banana and tomato fruit treated with CP or 1-MCP at 24°C remain insensitive for 12 days, then ripen normally. When treated with 3,3-DMCP, fruit remained insensitive for 7 days (Sisler et al. 1996b). Attempts to determine the period of insensitivity of growing pea seedlings after treatment with

Tab. 2. Concentration of 1-methylcyclopropene necessary for a maximum response.

Species	Response	Time, h	Concentration, nl l^{-1}
Musa sapientum	Ripening	24	0.7
Lycopersicon esculentum	Ripening	24	7.0
Dianthus caryophyllus	Senescence	24	0.5
Campanula carpatica	Display life	6	20.0
Pisum sativum	Growth	24	40.0
Vigna radiata	Abscission	24	40.0

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1-MCP have been inconclusive. The seedlings continued to grow for 3 days in the presence of ethylene, then stopped. The cells that were insensitive to ethylene probably had completed their expansion by this time, but may yet have been insensitive. Any newly-formed cells probably would have been sensitive since they would elaborate new binding sites.

Effect of temperature

At lower temperatures higher concentrations of the inhibitors are required for complete protection of carnation flowers against ethylene (Sisler et al. 1996a), suggesting that attachment to the receptor is less than at higher temperatures. The duration of the period of protection of tomato fruits by DACP is longer at lower temperatures (Sisler and Blankenship 1993b); the compounds probably are removed from the receptor during storage as this is a temperature-dependent process. It could also be that synthesis of new receptors is higher at higher temperatures.

Effect on ethylene production

Treatment of carnation flowers with 1-MCP prevented much of the climacteric rise in ethylene production (Sisler et al. 1996a) and treatment of *Phalaenopsis* flowers completely inhibited the pollination-induced increase in ethylene production (Porat et al. 1995a). In tomato fruit, DACP inhibited ethylene production, but after the fruits again became sensitive to ethylene the climacteric of ethylene production was considerably greater than that of control fruits. The greater capacity for producing ethylene may have developed during the period of receptor inactivation (Sisler and Blankenship 1993a).

Protection of ornamentals

DACP and 1-MCP have been used to render cut flowers and potted plants insensitive to ethylene. In rose, they protect against exogenous ethylene, increasing display life and reducing abscission of buds, flowers and leaves. These compounds (Serek et al. 1994a, 1996) provided as much protection as the conventional treatment with silver thiosulfate (STS). Similarly 1-MCP protected *Alstromeria, Antirrhinum majus, Consolida ambigua, Dianthus barbatus, D. caryophyllus, Matthiola incana, Penstemon hartwegii* x *P. cobaea* flowers (Serek et al. 1995a,c), *Chamelaucium uncinatum* (Geraldton wax flowers) (Serek et al. 1995b), *Phlox paniculata* flowers (Porat et al. 1995b), *Begonia* and *Kalanchoe* (Serek et al. 1994b), and *Petunia* flowers (Serek et al. 1995d).

Other responses of 1-MCP

In the study of the effect of 1-MCP on correlative effects of senescence, Serek et al. (1995d) showed that symptoms such as electrolyte leakage and lipid fluidity were also retarded in *Petunia*. Anderson et al. (1996) showed that 1-MCP treatment enhanced xylanase-induced ethylene production in tomato and *Capsicum annuum* leaves, perhaps by preventing feedback inhibition of ethylene biosynthesis. Cardinale et al. (1995) reported that 1-MCP inhibits ethylene epinasty in tomato plants. Reid et al. (1996) showed in *Kalanchoe* that higher concentrations of 1-MCP were required at lower temperatures, but found that there was no inhibition of ethylene responses in situations where ethylene production was already high in 1-MCP treated tissues. No reason for this curious observation was given. Lelièvre et al. (1997) showed that treatment with 1-MCP resulted in reduced accumulation of ACC oxidase transcripts and ethylene production during chilling. 1-MCP retards storage-induced leaf yellowing, but reduces rooting ability of stored cuttings (Müller et al. 1997).

Relationship between structure and activity

CP is a small molecule and because of its double bond it is planar, with only hydrogens projecting from the plane of the molecule (Fig. 2). It has a high amount of strain energy and strained compounds tend to bind to electron donor compounds such as low valent metals that act to relieve the strain. 1-MCP is also a planar molecule, with a methyl group attached at the double bond. This type of molecule is capable of an allene type of arrangement and this is probably also a factor in the bonding to the metal, assumed to be in the receptor. 3,3-DMCP has two methyl groups that are isolated from the double bond. Since the methyl group is relatively large when compared to hydrogen, these groups projecting from the plane of the molecule could result in a considerable steric effect. Methyl groups are also electron donors, which would lower the strain energy in the CP ring, and this may be a more important effect. Both steric effects and the effect of donating electrons (inductive effect) probably play a role in lowering the efficacy of 3,3-DMCP. These and other considerations should be taken into account in future development of further compounds designed to inhibit the effects of ethylene.

Radiolabel

There is evidence that ethylene binds to the receptor only in vivo. Any of these compounds should then be able to serve as a radiolabel in vivo and may help in the identifi-

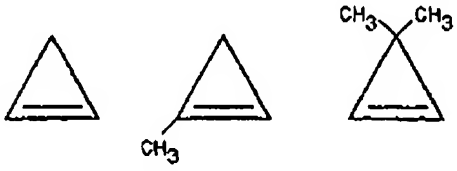

Fig. 2. Structures of cyclopropenes.

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cation of the ethylene receptor, perhaps leading to its eventual isolation. Some preliminary work has been done with 1-MCP as a radiolabel (Sisler et al. 1996a).

How do these compounds block the receptor?

1-MCP, CP and 3,3-DMCP presumably bind to a metal in the ethylene receptor. They would thus compete with ethylene for the receptor, preventing the latter from binding in treated tissues. This can be shown in two ways. The binding of radioactive ethylene to different binding components can be measured (Sisler 1991), and competitive action shown using a Scatchard plot (Dupille and Sisler 1995, Sisler et al. 1996a). It is also known that ethylene (and all three of the cyclopropene inhibitors) display apparent competitive interaction in a variety of tests such as banana and tomato ripening, flower senescence and pea seedling growth as shown by Lineweaver-Burk plots (Serek et al. 1994b). Results of these different approaches are in agreement with the hypothesis that these compounds compete with ethylene for binding to the receptor. After an ethylene treatment, much of the ethylene diffuses rapidly from the receptor, whereas 1-MCP (and other inhibitory compounds) remain bound for long periods (many days). While they are bound, ethylene cannot bind.

Why does 1-MCP not induce a response similar to ethylene? Ethylene may act by withdrawing electrons from a metal in the receptor, causing a ligand substitution process that induces an action response (Sisler 1977, 1991, Sisler and Goren 1981). 1-MCP should be capable of inducing such a response since, theoretically, it also would withdraw electrons from a metal. Since 1-MCP is so highly strained, its effect would be stronger than that of ethylene. As it binds to the receptor so strongly, it perhaps remains bound to the metal in the receptor, and the formation of an active complex is not completed, thus effectively blocking the receptor. As both ethylene and 1-MCP withdraw electrons they probably act similarly. We hypothesize that ethylene can leave the receptor, and that this departure is necessary for the formation of the active complex. Ethylene then would not be a part of the active complex, but the initiator of its formation. A model involving ligand substitution is proposed to account for the experimental observations (Fig. 3). Parts of this model have been presented before (Sisler 1977, 1991, Sisler and Goren 1981). Steps in the proposed model are: (1) ethylene approaches the metal and electrons are withdrawn; (2) another ligand in a trans position to it moves away from the metal; (3) yet another ligand moves toward the metal and as it does, ethylene is lost, and an active complex is formed; and (4) 1-MCP acts in a similar manner to ethylene, but it is not lost from the complex, and an active complex is therefore not formed. This model can account for many of the experimental results obtained in experiments with inhibitors of ethylene action. The proposed L_1, L_2, L_3, L_4, and L_5 ligands are unknown, but it is probable that one or more of them is localized on the ETR1 gene product, a protein thought to be part of the ethylene receptor (Schaller and Bleecker 1995). Alternatively, perhaps all of them are. Although unproven, this model may prove useful when designing experiments to elucidate the receptor's mode of action.

Conclusion

This review covers the major aspects of the new inhibitors at the receptor level, inhibitors that may be important new tools for controlling ethylene responses over extended periods. These compounds have only recently been discovered and much remains to be learned about them, but it is likely are they are capable of controlling all ethylene responses in plants.

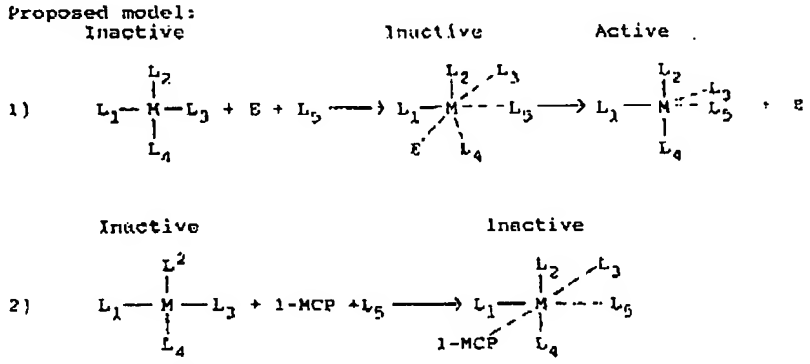

Fig. 3. Proposed model for action of ethylene and 1-methylcyclopropene (1-MCP) on the ethylene receptor. A metal (M) on the receptor surrounded by ligands (L_1–L_5) of unknown structure binds ethylene (E) or 1-MCP. With ethylene a ligand substitution process takes place and ethylene is expelled leading to an active receptor complex. With 1-MCP, it is too tightly bound to be expelled and an active receptor complex is not formed. This model can account for why ethylene is active and why 1-MCP blocks ethylene responses, but the details of this process remain to be shown.

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